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Direct comparison of evaporative light-scattering and condensation nucleation light-scattering detection for liquid chromatography

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Abstract

The analytical characteristics of a commercial evaporative light-scattering detection (ELSD) system were compared to those of condensation nucleation light-scattering detection (CNLSD) for the measurement of polyethylene glycol and polyethylene oxide samples with molecular masses ranging from 1000 to 45 000. Results for CNLSD with both an in-house constructed detector and a commercial condensation particle counter (CPC) were included in the study. Using a flow injection mode, limits of detection (LODs) for CNLSD were improved by up to a factor of over 1000 compared to ELSD. LODs with CNLSD for size-exclusion chromatography with an aqueous mobile phase were on average 130 times better with both the in-house detector and CPC, with average LODs of 15 ng/ml. While response for ELSD is typically nonlinear, the linear range for response of CNLSD was at least three orders of magnitude when the CPC was used for CNLSD, and for the in-house constructed detector when diffusion screens were employed. The polyethylene glycols tested in this study provided similar response on a mass basis with CNLSD, but 40% and 70% lower response was observed for a dextran and a polyacrylic acid, respectively.

Keywords: Detection, LC; Condensation nucleation light-scattering detection; Evaporative light-scattering detection; Poly(ethylene glycols); Poly(ethylene oxides)

1. Introduction

Detection for liquid chromatography (LC) can be accomplished by a variety of means. Detectors based on processes such as UV–Vis absorbance and fluorescence provide selective response for appropriate compounds and this response may extend to low concentrations. Others, such as refractive index (RI) detectors, provide more universal response, but only for moderately high concentrations. Mass spectrometers are sensitive and universal detectors, but are expensive and frequently not amenable to routine analysis. Currently, there are no simple, inexpensive LC detectors which can be considered sensitive and universal, and this absence is considered to be a limitation to many applications of LC [1,2].

With evaporative light-scattering detection (ELSD) for LC [3–6], the column effluent is converted to an aerosol (nebulized) that is subsequently desolvated (dried). Since analytes are generally much less volatile than HPLC solvents, conditions can be controlled such that the analyte does not evaporate, but remains behind as dry particles, while the mobile

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phase is removed. These analyte particles scatter light which can be monitored to provide detection of the analyte. Traditionally, these detectors are considered to have nearly universal response, but are also characterized as having modest limits of detection (LODs) (ca. 1 μg/ml), and nonlinear response. Although ELSD has been periodically described as providing response proportional to the mass of analyte present, differences in response due to density and refractive index effects are also known to occur [4].

With relatively pure mobile phases (<1 μg/ml residue after evaporation) where background is not the primary limitation, the LODs for ELSD can be considered with regard to particle size effects and light-scattering theory. The particle size of a desolvated aerosol particle can be related to the initial droplet size by:

$$D_D = D_I(C/\rho)^{1/3} \quad (1)$$

where D_D is the diameter of the desolvated particle, D_I is the initial droplet size, C is the non-volatile solute concentration and ρ is the solute density. Based on the droplet size distributions of typical pneumatic nebulizers for ELSD [5], and for analyte concentrations of 1 μg/ml, the largest desolvated particles that would be expected would be on the order of 100 nm in diameter and in low abundance; these particles are inefficient scatterers of radiation from practical light sources (e.g., UV–Vis) [7].

For detection of smaller particles, condensation particle counting (CPC) is an approach used in particle measurement technology, such as for determination of particle number concentrations in clean rooms [8]. With CPC, the flow of dry particles is mixed with a second flow of gas saturated with the vapors of a condensible fluid. This mixture of dry particles and saturated vapor is then cooled to supersaturate the mixture with respect to the vapor, causing condensation and growth of the dry particles from nanometer size to micrometer size droplets [9], greatly increasing the light-scattering intensity derived from the initial particle. Condensation of the vapor in the gas phase might be homogeneous, or heterogeneous (i.e., condensation onto particles), but higher supersaturation ratios are required for homogeneous nucleation, and the relative supersaturation ratio can be adjusted via temperature control to preclude homogeneous nucleation. Under conditions for heterogeneous nucleation, droplet formation will only occur in the presence of particles of sufficient size, and particles as small as 3–4 nm can be detected with nearly 100% efficiency [10].

Monitors for nonvolatile contaminants in solvents have been developed based on the atomization of the solvent, desolvation of the droplets and subsequent detection of the dry residue particles with a CPC [11–13]. More recently, we have explored the use of this approach, which we have termed condensation nucleation light-scattering detection (CNLSD), as a means of detection for chromatographic separations [14,15]. Using CNLSD in a flow injection mode, we have reported LODs for inorganic salts as low as the pg/ml level [14]. We have investigated the use of CNLSD with reversed-phase HPLC and found LODs for sulfanilic acid and sulfanilamide at the low ng/ml levels (ca. 3 ng absolute), and found response for a wide range of compound types to be nearly equivalent [15]. In the latter study, background impurities in the mobile phase were a limiting factor but solvent purification and/or diffusion screens could be utilized to reduce the effects of these impurities. Particles with diameters as small as 2–3 nm can be detected by this approach, and these sizes are comparable to single molecules with molecular masses above 10 000. Considering this aspect, Lewis and coworkers [16] have used a novel electrospray apparatus and condensation particle counting as a means for counting macromolecules separated by size-exclusion chromatography (SEC).

The goal of this work is to directly compare the detection capabilities of CNLSD and ELSD using a chromatographic system which provides a relatively low background for CNLSD and therefore an indication of the level of improvement that can be obtained under ideal conditions for CNLSD in its present configuration. We will describe results comparing the detection characteristics (LODs, linear dynamic range, etc.) of commercial ELSD and CNLSD. Measurements of a series of polymers were made both in a flow injection mode, and after separation by SEC. The effects of each detector on separation characteristics will also be described, as well as preliminary indication of the potential utility of CNLSD for molecular mass determinations by SEC.

2. Experimental

The CNLSD instrument employed for most measurements, which we will call CNLSD-1, was constructed in-house and has been described previously [14,15]. The instrument was operated with a nebulizer gas flow-rate of 1 l/min of nitrogen with the flow controlled using a Tylan (Torrance, CA, USA) Model 280 mass flow controller, and a saturator flow of 1 l/min of nitrogen controlled using a rotameter. A Meinhard (Santa Ana, CA, USA) Type C concentric pneumatic nebulizer was used for aerosol generation with an impaction plate located ca. 0.1 cm from the tip of the nebulizer. The impaction plate was located this close to the nebulizer to more efficiently remove large aerosol droplets, and thereby reduce background signal and noise levels. The aerosol was desolvated in a drift tube heated to 120°C, followed by a Friedrichs condenser cooled to 0°C.

For some experiments, the same aerosol generation and desolvation apparatus was used, but a TSI (St. Paul, MN, USA) Model 3025A CPC was used for particle growth and detection. In the text, we will refer to this system as CNLSD-2. The 3025A CPC can detect particles with diameters as small as 3 nm with high efficiency, and as many as 10^5 particles/ml.

For some experiments with CNLSD-1, diffusion screens were used to modify the dry particle size distributions, and alter the detector response. The diffusion screens employed in this study (Model 376060 particle size selector) were obtained from TSI. These devices act as particle filters that are selective for particles below some particular size, and are based on the fact that smaller particles have higher diffusion coefficients and are more likely to diffuse to and collect on the screen surfaces. The 50% cutoff diameter can be increased by increasing the number of screens [17]. When used, the diffusion screens were placed within the gas flow system as described previously [15].

The ELSD instrument tested was a Sedex (Paris, France) Model 55 operated with a nebulizer gas flow-rate of 2.5 l/min of nitrogen, and an auxiliary gas flow-rate of 0.5 l/min of nitrogen. This system differs from other ELSD systems in that the aerosol is sprayed into a room-temperature spray chamber and the aerosol that leaves the spray chamber is carried through a heated drift tube. With other designs [3–5], the aerosol is sprayed directly into a heated drift tube prior to the detector. The drift tube was maintained at a temperature of 41°C. These operating conditions were in accordance with the manufacturer's recommendations and provided optimum response.

Chromatographic separations were conducted using a 300×7.8 mm Phenomenex (Torrance, CA, USA) Polysep-GFC-P3000 SEC column, with a 75×7.8 mm guard column of the same material. The mobile phase was high-purity water obtained from a Barnstead (Dubuque, IA, USA) NANOpure water system and was controlled to 1 ml/min using an Alcott (Norcross, GA, USA) Model 760 HPLC pump. Sample volumes of 200 μl were injected using a Rheodyne (Cotati, CA, USA) Model 7125 injection valve.

Poly(ethylene glycol) (PEG) samples with average molecular masses (polydispersities in parentheses) of 1000 (1.10), 5000 (1.04), 11 000 (1.10) and 20 000 (1.06) were obtained from P.J. Cobert and Assoc. (St. Louis, MO, USA), and a poly(ethylene oxide) (PEO) sample of 45 000 (1.07) average molecular mass was obtained from Phenomenex. A 17 400 (1.54) molecular mass dextran, and a 2000 (ca. 2) molecular mass polyacrylic acid were obtained from Aldrich.

Most data were collected using a strip chart recorder. Chromatograms presented within this report were collected using the CPC controlled by a CompuAdd 486 personal computer. The data files were then transferred to a Macintosh Quadra 610 (Apple Computer, Cupertino, CA, USA) for final presentation using Kaleidograph (Synergy Software, Reading, PA, USA). LODs were calculated using a 3σ criterion [18]. Calibration plots for LOD calculations included standards down to concentrations which provided signal-to-noise (S/N) ratios$\leq$20, where S/N is the ratio of the signal level to the standard deviation of the background signal. For ELSD data, LODs were obtained by extrapolating the best-fit power curve down to a S/N equal to three [18], while a linear extrapolation of CNLSD data to this S/N was employed. S/N values were calculated using peak signals, and standard deviations of the background noise levels, which were calculated by

computer or manually estimated as 1/5 of the peak-to-peak noise level [19].

3. Results and discussion

3.1. Comparison of response for ELSD and CNLSD: flow injection

Fig. 1 provides representative data comparing the response, as *S/N* (as background noise) ratios, for ELSD and CNLSD-1, obtained for the 11 000 molecular mass PEG with samples introduced in a flow injection mode (FIA) (i.e., without a column). For this compound and the conditions employed, response for CNLSD extends to below the ng/ml level, while that for ELSD is limited to around the μg/ml level, as expected. In addition, the concentration response range for CNLSD covers about four orders of magnitude, compared to less than two orders of magnitude for ELSD. Some curvature in the plots for both CNLSD and ELSD is indicated. For all of the PEGs and PEO, response for CNLSD was comparable to that for the 11 000 molecular mass PEG. Calibration curves for ELSD plotted on a linear scale (Fig. 2) showed the typical nonlinear response for ELSD [20,21] characterized as a power curve, where $y=ax^n$, and exponents (n) varied between 1.6 and 1.8 for the four PEGs.

The left-most columns of Table 1 list LODs of both systems for data obtained via FIA. With CNLSD, the LODs in the FIA mode were calculated via extrapolation of the best-fit line down to an *S/N* of 3. For the five polymers tested, LODs for FIA with CNLSD-1 were at the 1 ng/ml level on average, while those for ELSD averaged 430 ng/ml. Improvements in LODs with CNLSD-1 were as high as a factor of 1300 and averaged 780.

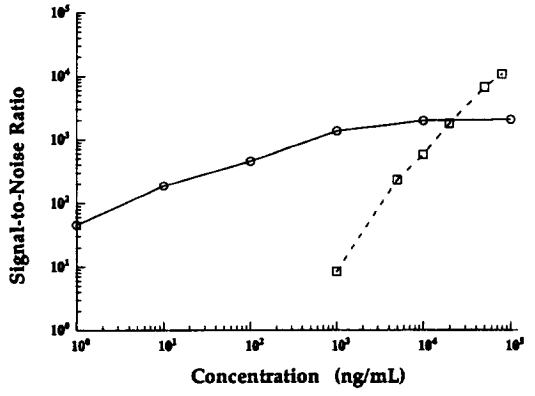

Fig. 1. Comparative calibration data for PEG 11 000 using (□) ELSD and (○) CNLSD-1.

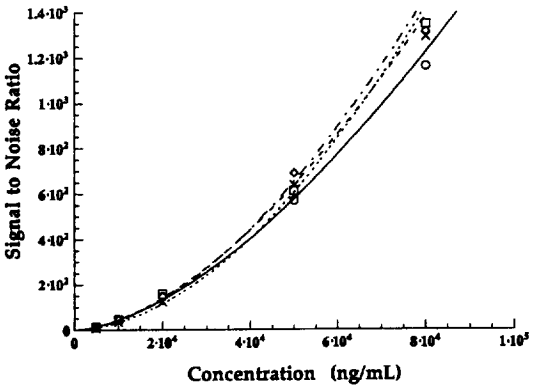

Fig. 2. Calibration plots on a linear scale of various PEGs for ELSD depicting the nonlinear response of this detector. Curves: ○=PEG 1000, $y=1.6769\cdot10^{-5}\cdot x^{1.604}$, $R=0.99972$; □=PEG 5000, $y=1.1531\cdot10^{-5}\cdot x^{1.648}$, $R=0.99983$; ◇=PEG 11 000, $y=5.4585\cdot10^{-6}\cdot x^{1.7193}$, $R=0.99698$; ×=PEG 20 000, $y=1.6667\cdot10^{-6}\cdot x^{1.822}$, $R=0.99771$.

Fig. 3 shows representative calibration data obtained using CNLSD-2 with FIA. In this case, linearity over the range from 1 to 1000 ng/ml is improved over that obtained with our laboratory-built detector, as exemplified by correlation coefficients which are greater than 0.999 for these examples and at least 0.99 for each of the polymers studied. *S/N* ratios for flow-injected samples with CNLSD-2 were slightly lower than those obtained with CNLSD-1, as indicated by the higher LODs for CNLSD-2 listed in Table 1. This may result from the fact that CNLSD-1 samples the entire sample aerosol flow, whereas the CPC (used with CNLSD-2) samples only about a third of the sample flow, discarding some the analyte and potential signal. The ratios of LODs for CNLSD-1 to CNLSD-2 averaged about 0.7. Improvements in LODs using CNLSD-2 compared to ELSD were as high as 800 and averaged 370.

With CNLSD (either detector) in this FIA mode, however, signal levels below the 50 ng/ml level (i.e., within a factor of 50 of the calculated LODs) were highly imprecise and curvature of the data indicative of background contamination was ob-

Table 1
LODs (ng/ml) for ELSD and CNLSD

Compound	FIA			SEC		
	ELSD	CNLSD-1	CNLSD-2	ELSD	CNLSD-1	CNLSD-2
PEG 1000	380	0.39	2.0	1900	17	15
PEG 5000	390	1.7	2.9	1600	21	
PEG 11 000	470	0.37	1.3	2200	14	23
PEG 20 000	480	0.77	0.6	2700	17	16
PEO 45 000		1.6	1.7		3.7	9.5
Average	430	0.97	1.7	2100	14	16

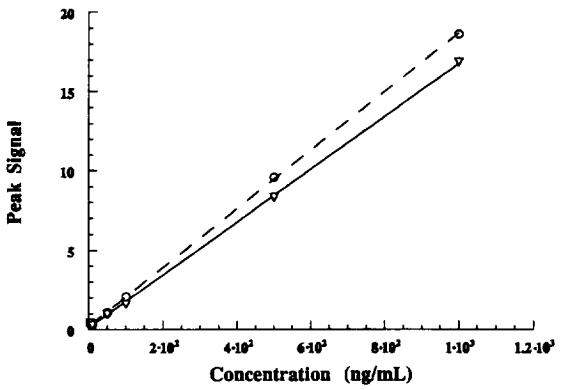

Fig. 3. Calibration plots on a linear scale for (▽) PEG 1000 ($y=0.10627+0.0167x$, $R=0.99974$) and (○) PEG 45 000 ($y=0.195+0.018534x$, $R=0.99993$) for CNLSD-2.

served, as previously reported by others during the development of this approach as a residue after evaporation monitor [11]. The FIA data in Fig. 4 depict this effect. This contamination is different than blank contamination since injections of pure water reproducibly provide no measurable signal.

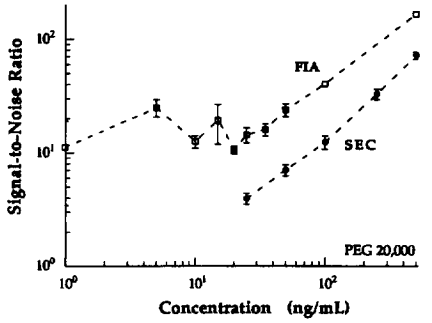

Fig. 4. Calibration data near the LOD for the 20 000 molecular mass PEG using CNLSD-2 with FIA (■) and after separation by SEC (○).

Standards preparation procedures using glassware appear be the primary source of this contamination as metal ions, silicates, etc., although since no selective response for nonvolatile contaminants can be obtained with CNLSD in the FIA mode, essentially any nonvolatile contaminant from any source can give rise to this background contamination. Assuming the development of adequate protocols for elimination of this background, extrapolation of the data using the linear portions of the calibration plots would provide an accurate estimation of LODs. The primary emphasis of this work is the application of this detection technique to HPLC, however, where in principle the separation will provide the required selectivity to distinguish these contaminants from the analyte based on differences in retention.

3.2. Comparison of response for ELSD and CNLSD: SEC

Clearly, LODs will be degraded due to dispersion with on-column injection and LODs with ELSD on-column are approximately five times higher than those for FIA as shown in Table 1. LODs for on-column injection with CNLSD-1 rose by, on average, a factor of fourteen compared to FIA. The reason for the larger degradation in LODs for CNLSD compared with the Sedex ELSD system is uncertain at this time but may be due to artifacts related to the contamination effects observed at low concentrations with FIA. The end result of this feature is lower levels of LOD improvement with CNLSD for on-column injection, averaging about 130 times better. Importantly, as shown by the SEC data of Fig. 4, close inspection of closely spaced calibration data to within a factor of two of the

LODs provides no evidence of the curvature due to contamination observed for FIA calibration data and R.S.D.s remain below the 20% level, indicating that these contaminant effects on calibration curves are alleviated by the selectivity provided by the separation process.

With CNLSD-1, the average LOD for the PEGs was 14 ng/ml, which is approximately 30 times lower than the average concentration LOD for three proteins ranging in molecular mass from 17 000 to 669 000 reported by Lewis et al., using their electrospray-CPC apparatus [16]. On the other hand, the absolute LODs for the PEGs reported herein range from 0.74 ng (or 16 fmol) for the 45 000 molecular mass PEO, to 3.4 ng (or 3.4 pmol) for the 1000 molecular mass PEG, or over two orders of magnitude higher than absolute LODs (0.9–9 pg) reported by Lewis et al. [16]. These differences arise from the fact that a microbore separation and a different aerosol generation system were employed in the latter work.

Unlike the case with the CNLSD-1, the background signal for CNLSD-2 increased when the column was placed on line. For FIA, background counts were 30–40 counts/ml, while with the column, levels ranged from 80–300 counts/ml. This probably results from the fact that the CPC used has the lowest threshold particle size for growth (3 nm) of those currently available, and this level is probably lower than that for our laboratory constructed detector. In fact, the variation in LODs from compound to compound listed in column 6 of Table 1 (9.5–23 ng/ml) followed the trend for the level of background measured during each data set (i.e., higher background levels gave higher LODs), rather than differences in signal levels. In this study, no particular effort was devoted to reducing this background; techniques which reduce this background contamination should lead to lower LODs. The average level of improvement on-column compared to ELSD was the same (factor of 130) for both CNLSD detectors.

Fig. 5 is a representative chromatogram depicting the separation of two of the PEGs. Notably, the concentrations of these two compounds are below the LODs of the ELSD, yet display high *S/N* ratios with CNLSD. Periodically, low-intensity peaks at ca. 6.5 and 16 min were observed and likely represent

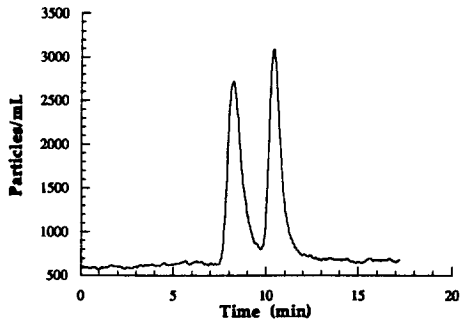

Fig. 5. Chromatogram depicting the separation of 1 μg/ml each of PEG 1000 and PEG 20 000 for CNLSD-2. The chromatogram was smoothed using an 11-point moving window average using Kaleidograph.

high- and low-molecular-mass contaminants, respectively, as were described affecting the reproducibility of signals for standards at low concentration with FIA.

Overall, average measurement precision for the various detectors was ca. 5% R.S.D. for ELSD, 6–7% R.S.D. for CNLSD-2, and ca. 9% with CNLSD-1.

Another factor of importance with a separation is the effect of the detector on chromatographic characteristics. For a sample concentration (10 μg/ml) which overlaps the calibration curves for ELSD and CNLSD, peak widths at half height for the PEGs and CNLSD averaged 36 s, compared to 35 s with ELSD. These values are within experimental error. In addition, Stolyhwo et al. [20] have previously reported that the nonlinear response of ELSD gives rise to an artificial increase in the resolution of the separation, because of the higher response per unit mass at higher concentrations. Correction for this effect as outlined by Stolyhwo [20] gives rise to peak widths for ELSD of 37 s, still within experimental error and indicating that dispersion is not significantly different for CNLSD and ELSD under the conditions utilized in this work.

3.3. Effects of the addition of diffusion screens to CNLSD-1 response

As indicated above, nonlinear response was observed for CNLSD-1. We have previously shown that background signals and the linearity of response of

this detector could be improved with reversed-phase separations through the use of diffusion screens [16]. Consequently, it was of interest to us to evaluate this approach with the chromatographic system employed in this study. Unlike the reversed-phase study, however, background levels resulting from column bleed and mobile-phase impurities in this study were low, and nearly equivalent to those that we might observe with FIA using ultrapure water, particularly when using the detector that we constructed in-house.

Fig. 6 compares the calibration plots obtained for a representative compound injected on-column as a function of the number of diffusion screens. The addition of a single diffusion screen results in a significant improvement in the linearity of response. The linear correlation coefficient was greater than 0.999 for the data obtained with one diffusion screen, covering a concentration range of over three orders of magnitude. The use of additional screens did not improve the linearity further, but only resulted in larger signal losses. Without diffusion screens, response with CNLSD depends on the overlap of the dry particle size distribution with that of the growth curve for the condensation and detection process. In effect, the diffusion screens act to change the particle size response curve for the detector system to larger size, and to the shape characteristic of the diffusion screen employed, as described by Lewis et al. [16]. Based on the data herein, the system response curve with the diffusion screens in control provides greater calibration linearity with CNLSD-1 than does the growth curve inherent to the detector.

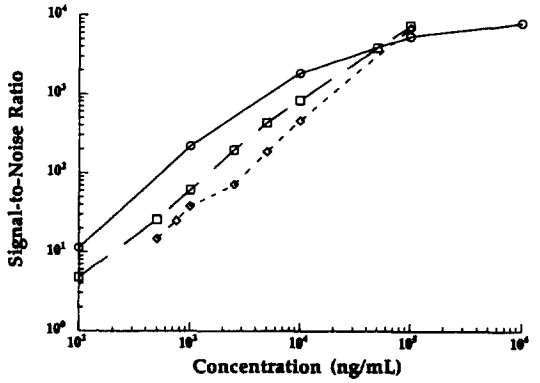

Fig. 6. Calibration plots depicting the effects of diffusion screens on response for PEG 11 000 with CNLSD-1. ○=No screen, □=1 screen, ◇=3 screens.

The loss of signal observed with the use of diffusion screens is expected since this process results in the removal/loss of some of the analyte. At the same time, the loss in signal would be expected to affect LODs. Table 2 lists LODs for the compounds tested in this study. On average, LODs increased by a factor of three with the addition of one diffusion screen, and by a factor of six with the addition of three. The use of diffusion screens therefore requires some tradeoff between LODs and linearity with CNLSD-1.

3.4. Variation in response from compound to compound

For use with SEC and molecular mass determinations, it would be advantageous if all compounds in samples provided essentially identical response per unit mass. With ELSD, light-scattering intensities can be affected by refractive index and density differences from one analyte to another [4]. In principle with CNLSD, droplets that are grown are nearly pure butanol (the condensing fluid), minimizing particularly the potential of effects due to refractive index differences.

The R.S.D. of the slopes of the lines for FIA obtained with CNLSD-2 for the different polymers used in this study was only 12% and no trend to response with regard to molecular mass was observed, indicating that the detector response is comparable for all of the polymers tested. Since this

Table 2
Effects of diffusion screens on LODs (in ng/ml and based on 3σ) for CNLSD-1

Compound	No Screen		1 Screen SEC	3 Screens SEC
	FIA	SEC		
PEG 1000	0.39	17	41	61
PEG 5000	1.66	21	20	111
PEG 11 000	0.37	14	61	81
PEG 20 000	0.77	17	87	171
PEO 45 000	1.62	3.7	22	
Average	0.96	15	46	106

FIA=results obtained for flow-injected samples (i.e., without a separation column); SEC=results obtained with size-exclusion chromatography using column specified in Section 2.

comparison is based on FIA, the slight differences in polydispersity from one polymer to the next could not influence the comparison. The differences in slopes of lines from compound to compound with CNLSD-1 were somewhat larger than those for CNLSD-2. The R.S.D. of the slopes of the lines obtained with the ELSD was slightly smaller, on the order of 7%.

In addition, relative response factors were determined for dextran and polyacrylic acid polymers in the FIA mode with CNLSD-2. In these cases, even larger differences in response than those between PEGs were observed, with the dextran providing about 60%, and polyacrylic acid providing only 30% of the average response for the PEGs. These results suggest that CNLSD can offer no improvement over ELSD with regard to being closer to an absolute mass detector. On the other hand, as reported previously [15], CNLSD does provide significant response for nearly all nonvolatile compounds tested thus far, as would be expected for a detector providing nearly universal response. With CNLSD, some factors which might be envisioned to cause differences in relative response from compound to compound include: (a) density differences which would influence the absolute sizes for the dry particle size distribution; (b) particle wettability (by butanol in this case) and particle morphology, which might influence the growth process; and (c) analyte specific factors which might influence particle coagulation or collection rates.

4. Summary and conclusions

These studies have shown that CNLSD has a number of advantages compared to ELSD as a detector for LC. LODs were found to be invariably lower, and in an FIA mode, as much as over three orders of magnitude lower with CNLSD. This characteristic should be useful when universal detection of trace species is required, such as for environmental applications. Calibration data for ELSD are not linear, while those for CNLSD can be linear for approximately three orders of magnitude. At the same time, ELSD appears to be more tolerant of mobile-phase contaminants than CNLSD. As such, this work using a high-purity mobile phase with low-bleed columns provides perhaps a favorable example of the improvements in detection that can be obtained with CNLSD in the configuration described within, although similar LODs were reported for reversed-phase HPLC [15]. We are currently developing newer versions of CNLSD (lower dispersion, higher efficiency, with improved aerosol characteristics), and protocols for preparation of high purity, low residue after evaporation solvents (e.g., acetonitrile, methanol, trifluoroacetic acid, etc.) for the demonstration of improved performance and application to other separation systems. With both ELSD and CNLSD, similar response on a mass basis was observed for the different molecular mass PEGs tested. Substantial differences in response for polymers of different types were observed with CNLSD, however.

Acknowledgments

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